DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO FILE
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Keywords

Potato, insect resistant (Colorado Potato Beetle), virus resistant (Potato Leafroll Virus), kanamycin resistance (nptII), *Bacillus thuringiensis* subsp. *tenebrionis* (BTT), 5-enolpyruvylshikimate -3-phosphate synthase (CP4 EPSPS), herbicide resistance

Background

In a submission dated July 17, 1997, Monsanto provided summary information to support their safety assessment of genetically modified NewLeaf Plus potatoes, specifically three lines NewLeaf Plus Russet Burbank RBMT21-129, RBMT21-152, and RBMT21-350 using nptII as a selectable marker and four lines NewLeaf Plus Russet Burbank RBMT22-82, RBMT22-186, RBMT22-238 and RBMT22-262 containing EPSPS as a selectable marker. This data was further supported by three previous summaries of NewLeaf potatoes. Potatoes containing the CryIIIA BTT protein have been on the market since 1995.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of potato is to confer tolerance to the Colorado Potato Beetle (CPB) and Potato Leafroll Virus (PLRV) in a single plant. Potatoes are grown to be consumed mainly as fresh vegetables. Potatoes are not in widespread use as an animal feedstuff (Memo-Nov. 25, 1997- Michaela Alewynse, CVM).

According to Monsanto, all seven lines contain the cyrIIIA gene which encodes the BTT protein (for resistance to CPB) and the PLRVrep gene from Potato Leafroll Virus (for resistance to PLRV). Three of their NewLeaf Plus varieties of potato have been modified to express neomycin phosphotransferase type II (nptII) (for selection) and the other four contain CP4 EPSPS for selection.

Regulatory Considerations

The use of the BTT protein and the PLRVrep gene protein as pesticidal substances and the use of the selectable marker, nptII, as a pesticidal inert ingredient in the development of the virus and insect resistant potato are under the regulatory purview of the Environmental Protection Agency (EPA). EPA regulates the use of the introduced genetic material encoding the viral coat protein, insect resistance protein, and the

selectable marker (including associated sequences required for expression) as well as the expression products. Therefore, in the consultation, we did not address the safe use of the viral coat protein and insect resistance protein as pesticides or the safe use of nptII in potato as a pesticidal inert ingredient. The main focus of the consultation with FDA was the compositional analysis of the transgenic potato as compared to the parental variety.

Molecular Alterations

Monsanto described the identity and function of the genetic material introduced into the potato lines by the *Agrobacterium tumefaciens*/Ti plasmid-mediated transformation system. The introduced genes were transformed into potato using two vectors PV-STMT21 and STMT22, derived originally from a standard plant transformation disarmed pTi58 plasmid.

It was reported by Monsanto that the three lines with kanamycin resistance, created using the PV-STMT21 vector have three chimeric genes introduced between the right and left border regions of the plant transformation vector. They included: 1) the chimeric gene for the selection of transformed plant cells which consisted of the promoter region of the nopaline synthase gene from the Ti plasmid of *A. tumefacients*, the nptII gene, and the nontranslated 3' region of the nopaline gene referred to as NOS 3'; 2) the chimeric gene responsible for the control of the CPB which consists of the *Arabidopsis thaliana* ribulose-1,5 -bisphosphate carboxylase small subunit ats1A promoter, the CryIIIA gene which encodes the BTT protein and the nontranslated 3' region of the nopaline synthase gene (NOS3'); 3) the chimeric gene responsible for control of PLRV which consisted of the 35S promoter region of the Figwort mosaic virus, the full length PLRVrep gene from a naturally occurring PLRV and the nontranslated 3' region of the pea small subunit of ribulose-1,5-bisphosphate carboxylase referred to as E9 3'.

In the case of the four lines created using the PV-STMT22 vector the introduced chimeric genes included: 1) the chimeric gene for selection of the transformed cells using glyphosate, which included the 35S promoter region of the Figwort mosaic virus, the CP4 EPSPS gene which encodes the enzyme 5 enolpyruvylshikimate-3-phosphate synthase and the nontranslated 3' region of the pea small subunit of ribulose-1,5-bisphosphate carboxylase; 2) the chimeric gene responsible for the control of the CPB which consists of the *Arabidopsis thaliana* ribulose-1,5 -bisphosphate carboxylase small subunit ats1A promoter, the CryIIIA gene which encodes the BTT protein and the nontranslated 3' region of the nopaline synthase gene (NOS3'); 3) the chimeric gene responsible for control of PLRV which consisted of the 35S promoter region of the Figwort mosaic virus, the full length PLRVrep gene from a naturally occurring PLRV and the nontranslated 3' region of the pea small subunit of ribulose-1,5-bisphosphate carboxylase referred to as E9 3'.

Expressed Protein

Three proteins are expressed in each of the lines. In theNewLeaf Plus Russet Burbank lines RBMT21-129, RBMT21-152, and RBMT21-350 the nptII protein, the CryIIIA CPB control protein and the PLRV ~~coat protein~~, are expressed. In NewLeaf Plus Russet Burbank lines RBMT22-82, RBMT22-186, RBMT22-238 and RBMT22-262 CP4 EPSSPS, the CryIIIA CPB control protein and the PLRV ~~coat protein~~, are expressed.

The nptII protein which is used as a selectable marker, was approved for use as a processing aid in several crops in 1994. Monsanto also received an exemption from the requirement of a tolerance for this protein as a pesticidal inert ingredient from the EPA on September 28, 1994 in all crops.

The CP4 EPSPS enzyme was isolated from *Agrobacterium* sp. strain CP4. The enzyme is in the shikimate pathway for aromatic amino acid biosynthesis in all plants, bacteria, and fungi but is not present in mammalian metabolic pathways. The EPSPS enzyme is normally inhibited by the herbicide glyphosate (N-phosphonomethylglycine). In contrast, CP4 EPSPS is not inhibited by glyphosate resulting in glyphosate tolerance. This gene product has been evaluated in previous FDA consultations for corn, cotton, and potato products.

Monsanto stated that the CryIIIA protein from *Bacillus thuringiensis subsp. tenebrionis* (BTT) introduced in these NewLeaf Plus potato varieties is identical to that found in nature and in commercial BTT formulations available since 1988. The EPA approved Monsanto's request for an exemption from the requirement of a tolerance for this protein in 1995.

According to Monsanto, the PLRV replicase protein confers resistance to the Potato Leafroll Virus. Monsanto presented evidence that virus-infected plants, including PLRV-infected potatoes, tomato, and pepper have been part of the human and domestic animal food supply without detectable adverse health effects.

Compositional Analysis

Monsanto presented data concerning the levels of total solids, sugars, vitamin C, soluble protein, and natural glycoalkaloid toxicants. Monsanto also performed proximate analysis and measured totoal protein, moisture, fat, ash, crude fiber, carbohydrates, and calorie content. Monsanto reported that the levels of all components analyzed were statistically identical to those in the control potatoes or were within the levels reported in the literature for commercially available Russet Burbank potatoes. These data allowed the firm to conclude that the composition of the NewLeaf Plus potato varieties are equivalent to the composition of traditional potatoes.

Conclusions

Monsanto has concluded the NewLeaf Plus potatoes containing the transformation are not materially different in composition, nutrition, and safety from potatoes currently grown, processed, marketed, and consumed for human food. At this time, based on Monsanto's description of its data and analyses, the Agency considers Monsanto's consultation on NewLeaf Plus potatoes to be complete.

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